



Original Research Article

Evaluation of efficacy of assay of lactate dehydrogenase in serum and pleural fluid in the diagnosis of tubercular pleural effusions

Shyam Prasad B. R¹, Thanuja Ramanna², Bhagyashree. K. Bhuyar^{3,*}

¹Dept. of Biochemistry, Govt Medical College, Anantapur, Andhra Pradesh, India

²Dept. of Biology, University of Memphis, Tennessee, USA

³Dept. of Biochemistry, Karwar Institute of Medical Sciences, Karnataka, India



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LDH - lactate dehydrogenase

ADA adenosine deaminase

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NAD nicotinamide adenine

dinucleotide

ABSTRACT

Tuberculosis is a communicable disease with differing manifestations. It is a disease which is one of the leading causes of morbidity and mortality worldwide and a disease which is affecting economy directly and indirectly. Extra pulmonary manifestations are an area of interest to laboratory as diagnosis is often difficult and not definitive. Pleural effusion is one of the commonest extra pulmonary manifestations. Differential diagnosis is a big palindrome for pleural fluids.

Aim: To test the reliability of lactate dehydrogenase of serum and pleural fluid in diagnosing tubercular effusions.

Materials and Methods: Serum and Pleural fluids of tubercular and non tubercular origin were assayed for lactate dehydrogenase and studied.

Results: The serum levels were sensitive and not specific and pleural fluid levels were neither sensitive nor specific.

Conclusion: The lactate dehydrogenase activity may be used a tool to distinguish exudates and transudates and also the ratio of lactate dehydrogenase and adenosine deaminase may be used if any other method are not available.

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1. Introduction

Tuberculosis is a disease caused by infection with mycobacterium tuberculosis. It can affect any organ but the lungs¹ are the most commonly affected due to its mode of spread by aerosol droplet type of infection and is called pulmonary tuberculosis. It can also affect other organs or tissues and is called extra pulmonary tuberculosis and pleural cavity is one of the commonly affected and manifests in majority of cases as pleural effusions.²

Tuberculosis is a disease of concern worldwide since many decades as it affects all ages and leads to morbidity and concomitant spread in active cases. Pulmonary tuberculosis can be diagnosed and confirmed by sputum examination or chest x – ray or other biochemical and

serological methods. Traditional methods of diagnosing tuberculosis do not recognise a significant proportion of tubercular cases of pleural effusions which are primarily immunologic process with a possible small number of tuberculosis bacilli.³ Consequently a majority of pleural fluids are negative to acid fast staining and culture for mycobacterium. A combination of invasive pleural biopsy culture and histology increases diagnosis a accuracy of tubercular pleurisy by 90%.⁴ This approach may require more than 6 samples of biopsy.⁵ But studies have shown that the sensitivity of pleural fluid cytology to be around 62%⁶ on an average.

Pleural effusion remains an area of research for investigation as cheaper and confirmative tests are required for early and easy diagnosis. Enzymatic studies with Adenosine Deaminase, Amylase, Lactate Dehydrogenase,

* Corresponding author.

E-mail address: shyamcapt@yahoo.com (B. K. Bhuyar).

Alkaline Phosphatase, etc are tried and tested by various research scientists to assess their efficacy in diagnostic arena in pleural fluid.

Lactate dehydrogenase⁷ is an Oxidoreductase, intra cellular enzyme with enzyme commission number 1.1.1.27.⁸ It is seen in Glycolysis pathway, for inter conversion of pyruvate and lactate with concomitant inter conversion of NAD and NADH for energy production. It is present in all cells. It is composed of two sub units and forms a tetramer and by arrangement of peptide chains, 5 isoenzymes – LDH-1, LDH-2, LDH-3, LDH-4, & LDH-5 are seen and can be separated by polyacrylamide gel electrophoresis. It is regulated by feed back inhibition.

Its levels are increased in haemolytic anaemia's, vitamin B12 deficiency, infections, infarctions, acute kidney disease, acute liver disease, rhabdomyolysis, pancreatitis, bone fractures, cancers of testicular origin, lymphomas, shock, hypoxia, tissue damage.

LDH as a pathophysiological marker has been studied in relation to several opportunistic infections, including infection by *Pneumocystis carinii* pneumonia, tuberculous and bacterial pneumonia,⁹ megaloblastic anaemia, acute leukemia, urinary tract infection, pulmonary embolism, pulmonary tuberculosis in adults, in tubercular & cerebrospinal meningitis¹⁰ and testicular tuberculosis,¹¹ reflecting its diagnostic utility in specific clinical cases. It may also serve as a sensitive marker of epidermal (skin) toxicity¹² earlier studies have shown that LDH is elevated in serum of tuberculosis cases more than non tubercular cases.⁷

2. Aim

This study was conducted to evaluate the utility of levels of activity of Lactate dehydrogenase in serum and pleural fluids in diagnosing tubercular pleural effusions.

2.1. Materials

The study was conducted at Government Medical College and Government General Hospital, Ananthapur. Institutional ethics committee approval was obtained for the study, methods, sampling methods and Proformas for conducting the study.

A total of 100 samples selected by purposive sampling method were evaluated. 50 tubercular and 50 non tubercular diagnosed by Chest X-Ray, pleural fluid analysis, Adenosine Deaminase and cytological studies were studied. Only new cases of pleural effusion were included in the study. Cases earlier diagnosed and or treated with anti tubercular therapy were excluded.

2.2. Methods

Samples of blood and pleural fluid were collected as per standard procedures of consent, aseptic collection and were timely transferred, stored and analysed in the laboratory

as per standard protocols. The samples were assayed for Lactate dehydrogenase using colorimetric kits with a semi auto analyser.

Results were tabulated in Microsoft office and statistical analysis was done with MEDCALC trial version software.

3. Results

The values obtained are as follows:

The mean Serum lactate dehydrogenase levels were 291.52 ± 71.725 in tubercular cases and 232.47 ± 116.356 in non tubercular cases with a t value of 3.102 and p value of 0.002 which is significant. The pleural fluids showed 251.602 ± 50.367 and 262.572 ± 71.716 in tubercular and non tubercular pleural fluids respectively with a t value of 0.960 and p of 0.339 which is not significant.

The values are shown in Table 1 and depicted in.

The odds ratio, Z statistic and significance level were as follows as shown in Table 2.

Receiver-operating-characteristics (ROC) analysis helped to decide the optimal cut off points for continuous variables based on their highest diagnostic accuracy. How well this system separates patients with and without tuberculosis was assessed by means of the area under the ROC curve, which ranges from 0 to 1, with 0.5 corresponding to no discrimination (i.e. random performance) and 1.0 to perfect discrimination. We also calculated sensitivity and specificity ratings for the derived score. P values less than 0.05 were considered to indicate statistical significance. All P values are two sided. Data were analyzed by using the MEDCALC statistical package.

The sensitivity, specificity, positive and negative predictive values for serum were 98%, 48%, 65.3% and 96% respectively. In pleural fluids it was 38%, 82%, 67.9% and 56.9% respectively as shown in Table 3. The ROC curves for serum and pleural fluid are plotted as Figures 1 and 2 respectively.

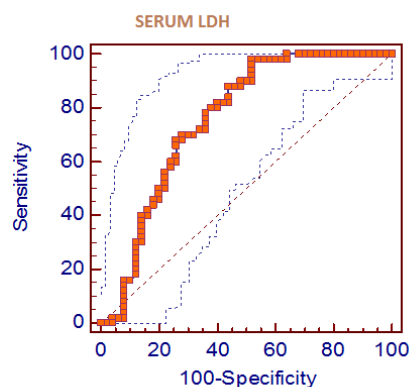


Fig. 1: ROC curve for pleural fluid LDH

Table 1: Analyte values obtained

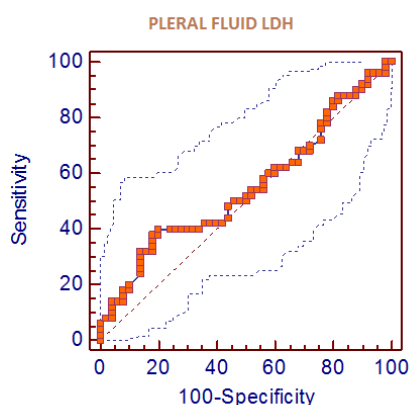
	Serum Mean	Standard deviation	t value	Pleural fluid Mean	Standard deviation	t value
Tuberculous effusions	291.52	71.725	3.102	251.602	50.367	0.960
Non tuberculous effusion	232.47	116.356	(p=0.002)	262.572	71.716	(P=0.339)

Table 2: ODDS ratio

Parameter	Odds ratio	Z Statistic	Significance level (AREA = 0.5)
Serum LDH	32.667	3.318	0.0009
P LDH	0.3267	0.680	0.4966

Table 3: Parameters of sensitivity, specificity

Parameter	Best cut off value (IU /L)	Sensitivity (%)	Specificity (%)	Area under ROC curve	Positive predictive value (%)	Negative predictive value (%)
Serum LDH	> 195	98	48	0.756	65.3	96
P LDH	< 218.6	38	82	0.547	67.9	56.9

**Fig. 2:** ROC curve for serum LDH

4. Discussion

A definitive diagnosis of Tubercular Pleural Effusion is made on the basis of the following criteria: (A) a positive AFB smear or positive cultures for *Mycobacterium Tuberculosis* in pleural fluid and pleural tissue; (B) chronic granulomatous inflammation in pleural tissue; and (C) a clinical response to antituberculosis treatment.^{13,14} However, in most studies, an ADA level ≥ 40 U/L in a lymphocytic exudate obtained via thoracentesis has been the most widely accepted indicator for a diagnosis of TPE.¹⁵

Lactate dehydrogenase, is an enzyme present in all cells. The LDH was thought to be elevated only in malignant pleural effusions but later on was also found in inflammatory etiology¹⁶ conditions also. LDH is an omnipresent intracellular enzyme which has been found elevated in a wide spectra of diseases due to tissue injury. It is

disproportionately high in sepsis and cancers and is a marker of poor prognosis.¹⁷ Further a high pleural fluid LDH has been implicated in poor prognosis in malignant effusions pleurodesis.¹⁸

Wang et al¹⁹ in their study on lactate dehydrogenase and adenosine deaminase levels in pleural fluids in diagnosis of tuberculous pleural effusion in a study done in 2017 in China did not find any increase in LDH levels in blood but found a good difference in ratio of LDH & ADA in pleural fluids between tuberculous and non tuberculous pleural fluids and concluded that LDH as a variable is not specific to tubercular pleural effusions, but LDH / ADA ratio can give a better diagnostic capability of tuberculosis.

Sharma et al in their study on LDH isoforms in TB [patients of Sahariya tribe⁷ in 2010, concluded that their study revealed a positive correlation between serum LDH level and the presence of mycobacteria and their load, suggesting utility of LDH as an important diagnostic marker of tuberculosis induced stress, at least in tribal areas lacking access to modern clinical tests.

Porcel et al in their study on a decision tree for differentiating tuberculous and malignant pleural effusion²⁰ on the utility of lactate dehydrogenase inferred that it can be used only for differentiating exudates from transudate using pleural fluid to serum LDH ratio and cannot be used as an independent diagnostic variable for tuberculosis.

Izidore S. Lossos et al.⁹ had observed in his study that LDH may be useful in the differential diagnosis of pleural effusion only when the diagnostic possibilities are limited to malignancy, pneumonia related effusion and CHF and not in tuberculosis. Richard W Light et al.²¹ has suggested that any condition causing an exudate pleural effusion can increase in the levels of pleural fluids and LDH alone as an enzyme cannot be used for the diagnosis of tuberculosis an

elevated pleural fluid LDH may present in TPE, PPE, and MPE, and the level is likely to range greatly from normal to extremely increased, which limits the use of LDH for identifying PPE in an individual patient due to the low sensitivity.¹⁹

Pleural fluid LDH is a frequently used biomarker to differentiate CPPE from UPPE, and a very high and isolated pleural fluid LDH level might be of specific diagnostic significance, especially for empyema.²² LDH in serum and pleural fluid was also elevated in malignant etiology pleural effusions in a study by ashish sharan et al.²³ My study is in agreement with the above studies and in line with the values obtained.

5. Conclusion

Lactate dehydrogenase even though elevated in serum and pleural fluid of tubercular effusions lacks specificity. The enzyme is found elevated in various diseases of inflammation, infection and malignancy. However LDH in association with ADA may be used in confirmation of diagnosis when other methods of investigation are not conclusive. It also can be used in differentiating between exudates and transudates. However further studies involving a large sample size with definitive conclusive etiology needs to be done to substantiate the findings.

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5.2. Abbreviations

TB- tuberculosis, LDH – lactate dehydrogenase, ADA- adenosine deaminase, ROC- receiver operating characteristics, NAD- nicotinamide adenine dinucleotide,

6. Conflict of interest

None.

7. Source of funding

None.

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Author biography

Shyam Prasad B. R Associate Professor

Bhagyashree. K. Bhuyar Assistant Professor

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